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Cyclic changes in amphibian egg microvilli occur during the division cycle: implication of MPF

C. AIMAR

Laboratoire d'Immunologie Comparée, Université Pierre et Marie Curie, CNRS UA 1135, 4 Place Jussieu, 75230 Paris Cedex 05, France

Summary

The microvilli (MV) of *Pleurodeles* (amphibian) eggs were examined following fertilization and compared with those of artificially activated eggs and enucleated eggs using scanning and transmission electron microscopy. The MV pattern in fertilized eggs was found to undergo a cyclic transformation during the course of the first few division cycles. Similar changes also occurred in the MV of artificially activated eggs and enucleated eggs. The reorganization of the MV was sensitive to cycloheximide and cytochalasin B, but was unaffected by colchicine. Thus, this MV alteration requires protein synthesis and microfilaments but microtubules are not implicated in this process. In addition, the effects on the MV pattern of the maturation or mitosis promoting factor (MPF) were tested. Injection of MPF into eggs at different times during the first division cycle nearly always induced an elongation of the MV. This observation suggests that MPF could regulate either directly or indirectly, *via* a MPF-sensitive factor, the cyclic transformation of amphibian egg MV.

Key words: amphibian, microvilli, egg cycle, cellular inhibitors, MPF

Introduction

When amphibian eggs are spawned, the egg surface is covered by microvilli (MV) which are easily observed by scanning electron microscopy (SEM). These MV undergo modifications during the different dynamic events associated with fertilization and cleavage. Remnants of MV are observed at the point of sperm penetration (Charbonneau and Picheral, 1983) and later at the site where the polar bodies are released (Elinson and Manes, 1978). Furthermore, in anurans, the general aspect of the MV is greatly modified during the release of cortical granules following egg activation (Elinson, 1980), and again during first cleavage (Monroy and Baccetti, 1975; Denis-Donini *et al.* 1976; Ohshima and Kubota, 1985). This latter observation has led to the suggestion that the MV are involved in cytokinesis during segmentation of the egg. Since the basic cytoskeletal component of both MV and the egg cortex is actin (Burgess and Schroeder, 1977), the MV may serve as a reservoir for this contractile protein or, alternatively, they may be a source of extra plasma membrane required for the formation of new plasma membrane following each cytokinesis (Sanger and Sanger, 1980).

Modifications of MV are part of the cyclic cytoplasmic modifications such as surface contraction waves and cortical contractions and relaxations that occur with the same basic rhythm as mitosis (Sawai, 1979; Hara *et al.* 1980). Such cyclic alterations are undoubtedly controlled by a cell cycle regulatory system which is thought to be governed principally by the maturation or mitosis promoting factor (MPF) (Masui and Markert, 1971).

MPF mediates the transition from the S-phase to the M-phase of the cell cycle. Its level rises and falls periodically during each division cycle (Gerhart *et al.* 1984). The presence of MPF coincides with endogenous phosphorylations (Karsenti *et al.* 1987; Labbé *et al.* 1988); due, in part, to the $p34^{cdc2}$ kinase, a subunit of the MPF in association with cyclin (Gould and Nurse, 1989; Gautier *et al.* 1990). The cytostatic factor (CSF), which stabilizes MPF in *Xenopus* eggs, is also present during a specific phase of the cell cycle (Newport and Kirschner, 1984; Masui and Shibuya, 1987). It can be hypothesized that MPF regulates directly or indirectly the modifications of the MV pattern during the division cycles of the amphibian eggs.

In the present paper, we describe the MV pattern in *Pleurodeles* eggs and examine possible factors modulating their form. (1) The morphology and distribution were initially characterized in fertilized eggs during the first division cycle by SEM. (2) To determine whether the modifications in the MV were controlled by cytoplasmic activity, independent of the nucleus, the same study was repeated on *enucleated* eggs following artificial activation. (3) The cellular mechanism controlling the MV pattern was investigated using specific inhibitors of protein synthesis (cycloheximide), actin polymerization (cytochalasin B) and microtubules (colchicine). (4) The level of MPF during divisions

cycles of fertilized and activated eggs was estimated *via* the cdc2 kinase activity. The effects on the MV of injection of MPF into activated eggs were then tested.

Materials and methods

Preparation of eggs

Pleurodeles waltl were provided by SEREA/CNRS, France. Fertilized eggs were collected after natural mating and unfertilized eggs were obtained from adult virgin females by an intraperitoneal injection of chorionic gonadotrophin (Organon, 500 i u.). After removing jelly coats manually using forceps, the eggs were maintained in a saline medium: diluted (1/10, vol./vol) in Steinberg medium (Steinberg, 1957).

Unfertilized eggs were activated by applying an electric shock (70 V, 80 μF) and then kept in the same buffer Eggs were enucleated within 10 min following activation using a fine tungsten needle and forceps (Aimar, 1988). For clarity, unfertilized, activated eggs and activated, enucleated eggs will be referred to as activated eggs and enucleated eggs, respectively.

Development of nucleated and enucleated eggs

At 20°C, the first division cycle of fertilized *Pleurodeles* eggs lasts about 6 h and subsequent cycles are 60 min. Similarly, activated eggs cleave 6 h after activation (PA) and exhibit a primitive cyclic furrowing activity for 3 or 4 h before lysing. As shown for other amphibians (Briggs and King, 1953), enucleated eggs do not divide.

To permit comparisons between different batches of eggs, the time of activation or fertilization is referred to as T=0, and the beginning of the subsequent cleavages as T=1.0, T=2.0 and T=3.0 Intermediate times are expressed as fractions of these periods

At different stages of the cell cycle, eggs (a minimum of 5 eggs per replicate) were fixed for SEM Eggs were examined hourly from the time of fertilization (or activation) up until the first division, and thereafter every 15 to 30 min up until 8 h PA. Some cortical fragments of eggs were also examined by transmission electron microscopy (TEM) at different times during the first cycle.

SEM and TEM

Eggs were fixed in 2.5 % (v/v) glutaraldehyde in 0 1 M sodium cacodylate buffer, pH 7.4 for 1 h at room temperature and then overnight at 10°C. Following fixation, the vitelline membranes were removed and eggs maintained in 0.1 M sodium cacodylate buffer, pH 7 4. For SEM, eggs were post-fixed in 1 % osmic acid, dehydrated through an ethanol series and then critical point dried and coated with gold.

These eggs were examined with a PHILIPS 515 SEM (CME of Institut Pasteur, Paris) or a JEOL JSM 840 A, operated at either 15 or 25 kV For enucleated and non-dividing eggs, the analyzed surface zone was limited to the region around the animal pole (<10°), while for cleaving eggs, the regions within 45° of the cleavage plane were examined Data were continuously recorded using a Panasonic video-recorder equipped with an image processing system (CRYSTAL; QUANTEL) and selected images were photographed.

TEM samples were processed at SCEM (University Marseille II) After postfixing eggs in osmic tetroxyde, small pieces of the egg cortex were cut from the animal hemisphere, stained *en bloc* in 1 % uranyl acetate for 30 min, dehydrated through an ethanol series and propylene oxide and then embedded in Spurr's resin (Fluka) Sections were cut at 80 nm, contrasted with uranyl acetate and lead citrate, and then observed with a Philips EM 400T electron microscope.

MPF preparations

Crude MPF was prepared according to the method of Dabauvalle *et al.* (1988) Non-activated *Pleurodeles* eggs were homogenized in MPF buffer (80 mM β glycerophosphate, 15 mM EGTA, 10 mM $MgCl_2$ and 1 mM DTT, pH 7.3). Each preparation (2 eggs in 2 μl of buffer) was used within 20 min.

Injections

150 to 200 nl of cytoplasmic extract containing MPF were injected into the animal hemisphere of activated eggs using a calibrated micropipette mounted on a Leitz manipulator. MPF was injected 15 min (T=0.04)· 1 h (T=0.16), 2 h (T=0.33), 4½ h (T=0.75) and 6 h (T=1 0) PA. These times were based on a division cycle of 6 h. The treated eggs were then fixed 45 min after injections. As controls, eggs were injected with MPF buffer (150 nl) at 15 min PA and fixed 45 min later In addition, uninjected eggs of the same age provided another control group.

Colchicine (C) was dissolved at 2 mg ml^{-1} in Tris buffer (100 mM) and 150 nl of this solution was injected 15 min PA Eggs were then fixed hourly up to 6 h and then at 6½ h and 7 h PA

Cytochalasin B and cycloheximide treatments

Cycloheximide (CH) and cytochalasin B (CB) were obtained from Sigma Chemicals. CB was prepared as a stock solution at 15 mg ml^{-1} in dimethylsulfoxide (DMSO) and used at 15 (or 30) μg ml^{-1} in full-strength Steinberg medium. Cycloheximide (100 μg ml^{-1}) and DMSO (30 μg ml^{-1}), used as control, were added directly to full-strength Steinberg medium. Eggs were treated with these inhibitors for 1 h periods following removal of the vitelline envelopes from activated eggs (20 to 25 eggs per replicate). Eggs were then fixed for electron microscopy between 30 min PA and 6 h PA.

Histone H1 kinase assay

MPF activity was shown to be correlated with the H1 kinase activity of its subunit $p34^{cdc2}$ (Gautier *et al.* 1989). This activity during the egg division cycle was measured according the technique developed by Jessus *et al.* (1990). Soluble protein fractions of egg cytoplasm were prepared in the MPF buffer then incubated 90 min at 4°C in presence of the 13×10^3 M_r protein (p13), encoded by the *suc1*+ gene (Brizuela *et al* 1987), conjugated to sepharose beds. cdc2–p13 sepharose pellets were then incubated 30 min with H1 histone (Boehringer) and [γ-^{32}P]ATP. The reaction was stopped by adding Laemmli sample buffer (1970) and boiling for 3 min Incorporation of γ-^{32}P into H1 histone was counted in a LS 7000 Beckman counter.

Control of MPF activity in crude MPF preparations and in MPF-injected eggs

The MPF activity in crude MPF preparations was controlled by injecting 150 nl of the homogenate into mature *Pleurodeles* oocytes In the presence of MPF, the germinal vesicle usually ruptures. To control the persistence of MPF activity into MPF-injected eggs, activated eggs were injected with 150 nl of the crude MPF preparation at respectivey 15 min and 1 h. Then samples of 3 eggs were tested for their histone H1 kinase activity every 10 min from the time of the MPF injection up to

50 min. Non-injected eggs and eggs injected with MPF buffer were used as controls.

Results

Morphological changes in the MV of fertilized, activated and enucleated eggs

(1) Fertilized eggs

Before fertilization, the egg surface was covered with many hemispherical or conical MV, diameters of 0.25 to 0.35 μm. They had a uniform distribution with an average density of 20 MV per μm^2 (structural type I; Figs 1A and 2A). Some eggs had finer and longer MV arranged in circular tufts about 1 μm in diameter. These MV usually disappear within 2 to 3 h after fertilization.

About 1 h after fertilization (T=0.16), the size of the MV began to decrease (0.20 to 0.30 μm in diameter), but their density increased (25 MV μm^{-2}). Next, ridges, 0.5 to 1 μm in length, were observed, and they appeared to form by MV extension and by fusion of neighbouring MV (Fig. 2B). This transformation occurred progressively. It was very evident by 2 h PA (T=0.32) and largely completed between 3 and 4 h PA (T=0.50 to T=0.66). By this time, the egg surface was organized in a discontinuous network of elongated and convoluted crests in which only a few dispersed globular MV (5 MV μm^{-2}; diameter, 0.20 to 0.75 μm) could be seen. Between the ridges of this network, deep depressions, often greater than 1 μm in diameter, occurred. These surface structures gave the egg a characteristic sponge-like aspect, (designated type II), in both SEM and TEM (Figs 1B, 2C).

The network of ridges gradually regressed from 4 h PA (type III; Fig. 1C). After 5 h PA (T=>0.80), the surface of the egg was mostly smooth, interrupted by periodic globular MV and some flattened MV crests (type IV; Figs 1D, 2D). Circular tufts of MV similar to those observed at the beginning of the cycle also reappeared. This pattern remained until the formation of the first cleavage furrow at 6 h PA (T=1.0).

At the beginning of the second division cycle (T=1.0 to T=1.25), very long MV, either pointed or finger-like in form, lined the edges of the cleavage furrow (type V, Fig. 1E). Similar changes at this stage have been observed in other amphibian species (Monroy and Baccetti, 1975). In areas away from the furrow, the extended MV slowly decreased in size and disappeared as the cycle progressed. By the end of the second cycle, only a few lobed MV (5 to 10 MV/10 μm^2) were scattered on a relatively smooth egg surface (type IV). The second cytokinesis at T=2.0 usually began immediately after the first cleavage furrow was fully completed. Once again, elongated MV appeared along the border of the new furrow.

About half-way through the third division cycle (T=2.5), the surface again consisted of extended MV and cavities (type II, III). At the completion of the third cycle, at 8 h PA (T=3.0), the egg surface resembled the surface at the end of the previous cycle (type IV). Furthermore, the same phenomena, consisting of MV extension followed by regression of the MV in the animal hemisphere then formation of elongated MV along the edges of the furrows at the beginning of cytokinesis, was repeated during the first three cleavage cycles

(2) Enucleated and activated eggs

At 30 min PA, the surface of activated eggs and enucleated eggs, with the exception of the site where the nucleus was removed, consisted of a type I MV pattern, like that observed in fertilized eggs at the same stage. However, the timing of their subsequent transformation was slightly different. The increase in MV size and the confluence of the MV occurred more slowly. In addition, after T=0.66, disorganization of the network of type II MV crests (Fig. 1F) was faster than in fertilized eggs. As a consequence, at T=0.80, these eggs resemble fertilized eggs at T=0.90 (Fig. 1D). At this time, rare globular elements (0.25 μm in diameter) and flattened MV crests (less than 10 MV/10 μm^2) were dispersed on a smooth egg surface (structural type IV). By 6 h PA (T=1.0), activated eggs divided while enucleated eggs did not divide. At the apex of enucleated eggs and at the edges of the pseudofurrows of activated eggs, elongated, finger-like type V MV developed (Fig. 1G).

During the period corresponding to the second division cycle of fertilized eggs (6 to 7 h PA), the surface of most activated eggs and enucleated eggs went through alternate phases of MV expansion and MV regression. At 6½ h PA (T=1.5), a relatively dense distribution of MV crests (type II and III), 1 to 3 μm in length, was present (Fig. 1H). At 7 h PA, (T=2.0), this network was replaced by small granular MV and some elongated MV (Fig. 1I).

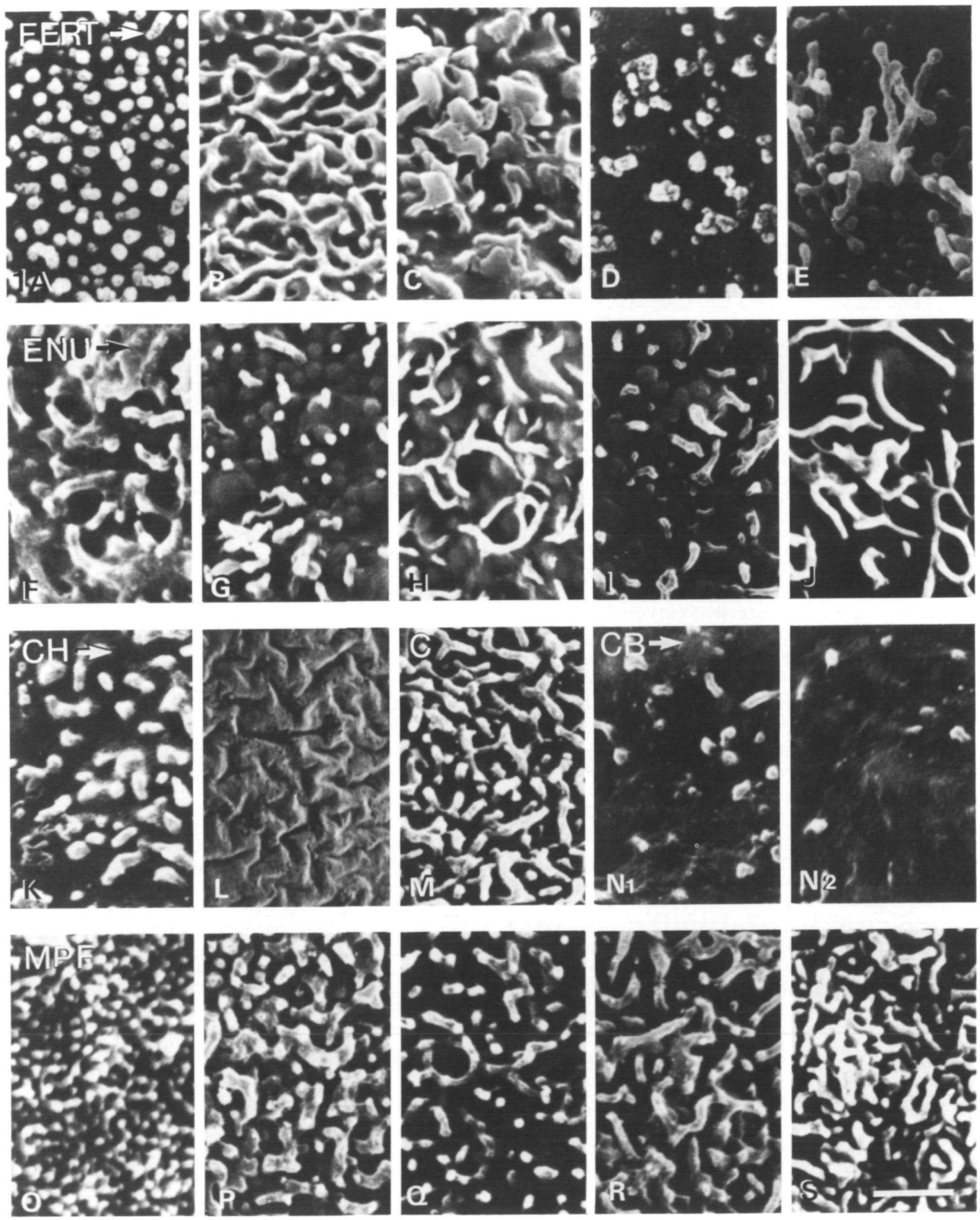
The same transformation in the MV, with the typical MV expansion (Fig. 1J), was repeated between 7 and 8 h PA, during the third division cycle in fertilized eggs. Thereafter, the surface of the eggs degenerated in a manner typical of cytolysis.

Effects of cellular inhibitors on the cyclic changes in MV of activated eggs

(1) Cycloheximide

When activated eggs were treated with cycloheximide, an inhibitor of protein synthesis, furrowing activity was completely blocked. Furthermore, cycloheximide limited the elongation of MV and accelerated their regression.

Although at 2 h PA, the network of lobed and convoluted MV and the depressions in the plasma membrane were well formed, by 3 h PA (T=0.50) the size of the MV was already decreasing, thereby creating shorter, dispersed MV crests (1 to 2 μm in length) (Fig. 1K). New individual MV (0.25 to 0.50 μm in cross section) were also evident. At the time when control eggs cleaved, the surface of cycloheximide-treated eggs became convoluted (Fig. 1L) and an apparently strong



constriction occurred at the marginal zone. Eggs degenerated without forming a cleavage furrow.

(2) Colchicine

Treatment of activated eggs with colchicine, which prevents depolymerization of microtubules, had no detectable effect on the evolution of MV during the first two division cycles (0 to 7 h PA). The treated eggs, like controls, underwent two successive cycles with alternating phases of MV extension and regression with the

Fig. 1. MV transformations in the animal hemisphere of *Pleurodeles* eggs as observed by SEM. (A–E) MV pattern during the first division cycle of fertilized eggs (FERT). Conical MV of structural type I at time T=0 0 (A); extended MV of type II at time 0.66 (B); convoluted MV of type III at time 0.83 (C), regressed MV of type IV at time 0.90 (D); finger-like MV of type V on the edges of the 1st furrow at time T=1.0 (E). (F–J) Periodic alternance of extended and regressed MV in *enucleated* eggs (ENU) MV elongation at times. T=0 33 (F); 1.50 (H) and 2.50 (J) and MV regression at times T=1.0 (G) and 2.0 (I) occur at the same times as in fertilized eggs. (K–N) Limited extension of MV of activated eggs treated with cycloheximide (CH) at T=0.50* (0.00)† (K, compare with B) and time T=1.0 (L). Normal MV pattern of eggs injected with colchicine (C) observed at T=0.16 (0.04) (M). Drastic alteration of MV treated with cytochalasin B (CB) at time T=0.60 (0.00) (N1 and N2; compare with B). (O–S) MV transformations in activated eggs injected with crude MPF preparation. Control experiment with MPF buffer at T=0 16 (0.04) (O). Injections of MPF provoke a rapid and characteristic MV extension at times T=0 16 (0.04) (P, to compare with O); T=0.29 (0.16) (Q) and T=0 81 (0.75) (S; compare with D). At time of treatments, the level of endogenous MPF of the eggs was low or decreasing. A normal MV extension was observed on MPF injected eggs at T=0.46 (0.33) (R), when level of endogenous MPF was higher. Bar=2 μm. Magnification, ×8000 except L. ×80.
* Time of egg fixation, † time of treatment.

normal rhythm (Fig. 1M) Nevertheless, these eggs degenerated at the end of the second division cycle.

(3) Cytochalasin B

The cytoskeleton of MV consists of actin filaments and polymerization of actin is inhibited by cytochalasin B (CB) (Brown and Spudich, 1981). In two series of *Pleurodeles* eggs, CB induced a rapid change in the MV, similar to that described in *Xenopus* eggs (Denis-Donini *et al.* 1976). Already at T=0.20, the density of spherical MV was markedly reduced (10 MV μm^{-2}, compared with 20 to 25 MV μm^{-2} in controls) and their elongation remained limited. By T=0.60, the evolution of the MV was well in advance of controls, and CB-treated eggs all had shorter MV (Fig. 1 N1,N2) As well, the MV network in treated eggs was much looser and did not include deep surface grooves. Some treated eggs did not have any MV on their surfaces. This latter phenomenon was accentuated at T=1.0, when 2/3 of the eggs did not have MV and the rest exhibited zones without MV and regions containing degraded MV. With CB treatment, no cleavage was observed.

Activated eggs incubated in DMSO displayed thick-crested MV and depressions at T=0.40, similar to the pattern observed in control fertilized eggs. At T=0.80, the MV network was reduced and, at T=1.0, fine elongated MV characteristic of cleaving eggs appeared. In contrast with CB-treated eggs, eggs incubated in DMSO exhibited pseudocleavages at the same time as control eggs.

Role of MPF on the cyclic activity of the MV

(1) Oscillations of histone H1 kinase activity during division cycles of Pleurodeles *eggs*

Upon fertilization, H1 kinase activity abruptly fell 5-

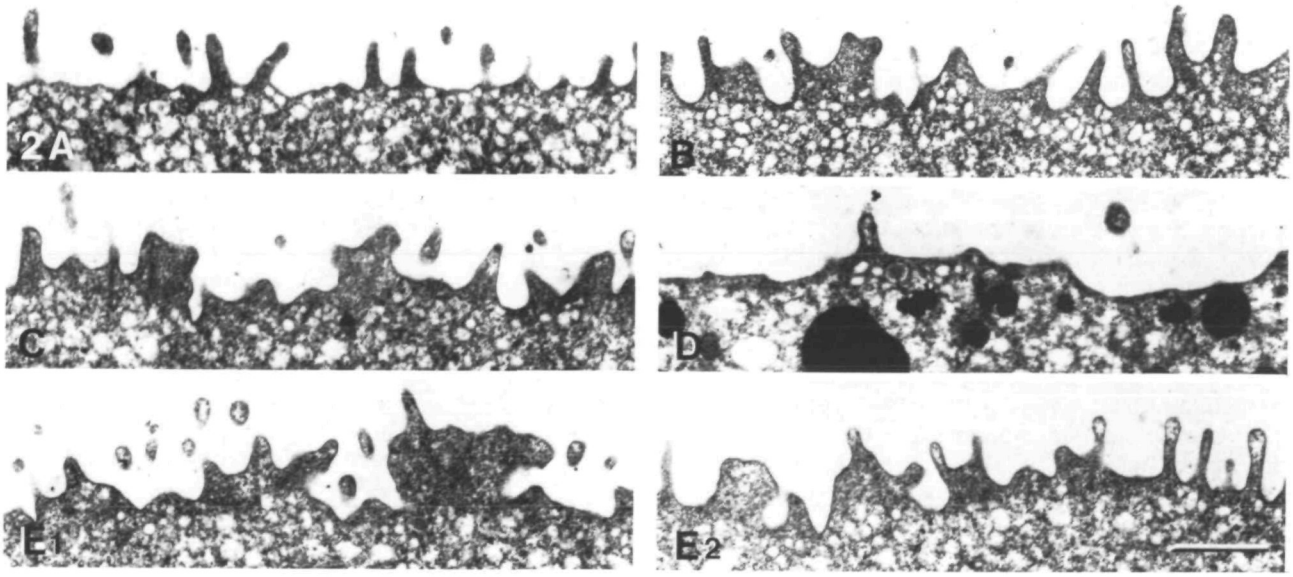


Fig. 2. Transformation of MV and the cortex in the animal hemisphere of *Pleurodeles* eggs as observed by TEM. (A–D) Cortex of fertilized eggs at different times after insemination: T=0 04 (A); T=0.30 (B); T=0.65 (C); T=0.95 (D) Note the progressive formation and elongation of ridged MV (type II) in B and C during this 1st cycle and the regressed MV of type IV (D) at the end of the division cycle (E1, E2) Cortex of artificially activated eggs injected with MPF at T=0.04 and fixed at T=0.16. The injection of MPF provoke the formation of elongated MV. Compare E1 and E2 with B. Magnification, ×5600 Bar=2 μm.

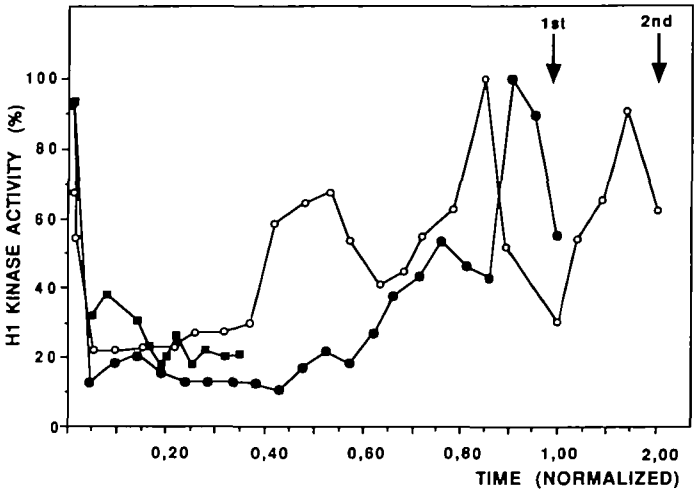


Fig. 3. H1 histone kinase activity during division cycles of *Pleurodeles* eggs White circles, fertilized eggs; black circles, activated eggs, black squares, activated eggs injected with crude MPF. The 100% H1 kinase activity level corresponds to 0.7 pmoles μg^{-1} of histone/20 min

fold and remained at a minimum level for about 2 h. At T=0.35 it increased, in two steps of equal durations, about one hour each, up to a maximum at T=0.80 (Fig. 3). H1 kinase activity returned to the minimum level, corresponding to 20% H1 kinase activity, at the time of the first division. The same cyclic activity, with a maximum at T=0.80, was observed during the second division.

Oscillations of H1 kinase activity in activated eggs were slightly different to those of fertilized eggs (Fig. 3). After activation, the kinase activity decreased 10-fold and a low level of activity remained constant till to T=0.40. After a two-stepped increase, the H1 kinase activity reached a maximum at T=0.90 of the cycle. As in fertilized eggs, it returned to a lower level at the time (T=1.0) of the first cytokinesis.

Kinetics of H1 kinase activity in fertilized or activated *Pleurodeles* eggs differ, in some parts, from that observed in *Xenopus* eggs (Gautier *et al.* 1989). The presence of a plateau of low kinase activity during the first third or more of the first cycle, then the stepped increase of H1 kinase activity, were not described for *Xenopus* eggs. These differences could be due to the very accurate examination of the kinase activity in *Pleurodeles* eggs. Egg samplings were done every fourteen minutes for a six hour division cycle, whereas, for *Xenopus*, they were done every ten minutes for a 1½ h cycle. Alternatively these differences could be the expression of species specificities.

(2) Effects of cytoplasmic extracts of MPF on the MV cycle

As shown in Fig. 3, following the injections of crude MPF, activated eggs maintained H1 kinase activity for 25 min, to a level about three times higher than that of the activated eggs used as controls. After that, H1 kinase activity decreased but remained always twice as high compared to controls. Injections of cytoplasmic extracts into full-grown *Pleurodeles* oocytes provoked germinal vesicle rupture in more than 50% of the cases, attesting the presence of active MPF.

This crude MPF provoked a very rapid rearrangement on the egg surface when injected during certain phases of the first division cycle of activated eggs. Following injection of active MPF at T=0.04, the eggs formed a network of elongated MV (type II or III, Figs 1P and 2E) by the time they were fixed (T=0.12 or 0.16; about 45 min PA). In contrast, most uninjected eggs and those control eggs injected with MPF buffer still presented the initial pattern of conical MV or were just beginning to show signs of MV alterations (Fig. 1O). MPF also advanced MV transformation when MPF was transferred at T=0.16. When such eggs were subsequently fixed at T=0.29, a dense type II network of MV crests was already present (Fig. 1Q), whereas in control eggs this network was just forming.

When MPF was injected when the MV were regressing (type IV, T=0.75), these eggs displayed a type II or III pattern, characterized by well-developed, convoluted MV, when fixed at T=0.81 (Fig. 1S). At this time, type IV MV were present on control eggs (Fig. 1D). It appears that MPF, at this stage, promotes the maintenance of extended MV. Lastly, when eggs were injected at T=1 and fixed midway through the second cycle (T=1.5), only a slight increase in the usual MV extension was noted in 2/3 of the injected eggs.

In contrast, in eggs that were injected with MPF at T=0.33 and fixed at T=0.46, (when MV were normally elongating) a type II network, like that of control eggs, was observed (Fig. 1R).]

Discussion

Cyclic activity of microvilli

The MV of *Pleurodeles* eggs have been shown to undergo a continual transformation from fertilization to first cleavage. Two principal phases were defined: one characterized by a network of large MV well formed at T=0.40 and which persisted about until T=0.70, and a second, which involved the disassembly of this MV network, taking place between T=0.70 and T=1.0. This extension of MV midway through the division cycle and their regression during cytokinesis were reproduced during subsequent division cycles.

In fertilized or parthenogenetic *Pleurodeles* eggs, the rhythm of the MV transformation was identical to that of the division cycle. Eggs experimentaly deprived of their nuclei and which never divide, exhibited similar MV changes, including the cyclic formation of finger-like MV that characterize cleaving amphibian eggs (Denis-Donini *et al.* 1976; Oshima and Kubota, 1985). These results suggest that the cyclic changes in the MV, as many other events that affect the egg surface, as cortical contractions or surface contractions waves (Sawai, 1979; Hara *et al.* 1980), are regulated by cytoplasmic factors independently of nuclear control.

These MV transformations in *Pleurodeles* eggs appear to be quite different from those occurring in

anurans. In *Rana* and *Xenopus*, an increase in MV size is observed immediately after cortical granules are released (Elinson, 1980; Charbonneau and Picheral, 1983), but there has been no description of a subsequent formation of a transient network of large MV. In these species, the MV exhibit no noticeable changes until the end of the first division cycle (Monroy and Baccetti, 1975; Elinson, 1980).

In anurans, the precocious modification of the MV appears to be due to a reorganization of the plasma membrane following exocytosis of cortical granules (reviewed by Grey *et al.* 1974). As in some other species (see Schroeder, 1981), urodeles do not usually have cortical granules (Picheral, 1977). The later extension of MV in these eggs, during the mid-phase of the cell cycle, may correspond to construction of new plasma membrane. The sensitivity of this MV elongation to cycloheximide coupled with the fact that protein synthesis occurs during this period (phase G) of the cell cycle argue in favour of this hypothesis.

Regulation of the MV pattern

Cycloheximide blocks amphibian eggs in interphase. It provokes nuclear anomalies and arrests mitosis (Sentein, 1981), as well as altering protein biosynthesis by limiting endogenous phosphorylation (Karsenti *et al.* 1987). Although protein synthesis is usually a prerequisite for the formation of new MV and plasma membrane that occurs prior to each division (Graham *et al.* 1973; Schroeder, 1981), in cycloheximide-treated *Pleurodeles* eggs, the first division was completely blocked, but a limited MV elongation did occur. Furthermore, the direct injection of calcium, ionophores and polyamines also leads to a rapid alteration in the egg surface, including MV elongation (Ezzel *et al.* 1985; Grant *et al.* 1984). That suggests that the modifications of MV size and form during the cell cycle are not strictly dependent on protein synthesis.

The astral microtubules associated with the centrioles play an essential role in the process underlying cell division (Sluder *et al.* 1986). A cyclic activity of asters involving assembly and disassembly of astral rays accompanies each division cycle (Inoue, 1981; Mitchinson, 1984), and contacts are established between the astral microtubules and the plasma membrane during each mitosis (Asnes and Schroeder, 1979). When centrioles are inactivated in eggs of a number of different amphibian species, division does not occur (Briggs and King, 1953; Hara *et al.* 1980; Karsenti *et al.* 1984; Aimar, 1988). However, colchicine treatment of microtubules inhibited division of *Pleurodeles* eggs but had little effect on the evolution of the MV. Thus, although microtubules are involved in furrowing activity, there does not appear to be a direct relationship between the aster cycle and the cyclic events controlling the plasma membrane of the egg.

On the other hand, CB prevented the cyclic changes in MV in *Pleurodeles* eggs as it does in *Xenopus* eggs (Denis-Donini *et al.* 1976), provoking rapid MV regression. CB acts by inhibiting actin polymerization, (Brown and Spudich, 1981) and has been shown to cause microfilament disorganization in the cortex of amphibian eggs (Luchtel *et al.* 1976; Franke *et al.* 1976). Moreover, Ezzell *et al.* (1985) have reported that *N*-ethylmaleimide-modified heavy meromyosin, which inhibits actomyosin-dependent processes, prevents MV elongation in *Xenopus* eggs. These different data provide evidence that the modifications of the pattern of MV involve actin rearrangements.

The cyclic activity of the MV implies the existence of a regulatory system and, in the case of amphibian eggs, has previously been attributed to MPF (Masui and Market, 1971). In *Pleurodeles* eggs, there was a strong correlation between the presence of MPF attested by the H1 kinase activity, and the extent of MV elongation. In fact, H1 kinase activity increases in two steps during the first division cycle. In subsequent division cycles, the kinase activity reaches maximal level shortly before cytokinesis. The period when MV were extended, at mid-phase of egg cycles, corresponds closely to the time of the first burst of H1 kinase activity during the first division cycle and to the period of high H1 kinase level during the following cycles.

In addition, the present study shows that injection of crude MPF at the beginning when level of endogenous MPF was low provoked, in a short time (less than 25 min), the formation of elongated MV. At the end of a cycle, when MPF level was decreasing, it favoured the maintenance of MV elongation. However, at the time of the first burst of H1 kinase when a high level of MPF is naturally present, the addition of exogenous MPF did not amplify the elongation of the MV.

Data on the variations of H1 kinase activity and their correlation with the cycle of MV and experiments on the effects of injections of MPF on MV pattern, provide further results implicating MPF or an MPF-derived product in the regulation of MV.

That regulation appears to be one of the earliest and rapid effects of MPF activity since MV elongation takes place at the beginning of the MPF amplification.

The consequence of the MPF activation at the G_2–M phase seems to be a large increase of protein phosphorylations (Maller, 1987; Adlakha *et al.* 1988) provoking a cascade of various events. One mitotic-specific phosphorylation was recently shown to affect caldesmon, a protein bound to the cortical actin (Yamashiro *et al.* 1990). It seems likely that MPF acting as a pleiotrophic agent, could affect the interactions between constitutive proteins of the MV promoting the transformations observed during the egg division cycles.

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